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During the years of the journal's publication, the Butler University Botany Department had an active program of research and student training. 201 bachelor's degrees and 75 master's degrees in Botany were conferred during this period. Thirty-five of these graduates went on to earn doctorates at other institutions.

The Botany Department attracted many notable faculty members and students. Distinguished faculty, in addition to Cain and Friesner , included John E. Potzger, a forest ecologist and palynologist, Willard Nelson Clute, co-founder of the American Fern Society, Marion T. Hall, former director of the Morton Arboretum, C. Mervin Palmer, Rex Webster, and John Pelton. Some of the former undergraduate and master's students who made active contributions to the fields of botany and ecology include Dwight. W. Billings, Fay Kenoyer Daily, William A. Daily, Rexford Daudenmire, Francis Hueber, Frank McCormick, Scott McCoy, Robert Petty, Potzger, Helene Starcs, and Theodore Sperry. Cain, Daubenmire, Potzger, and Billings served as Presidents of the Ecological Society of America.

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THE DIRECT MICROSCOPIC ANALYSIS OF MILK OVER A PERIOD OF ONE YEAR FROM THREE CREAMERIES SERVING INDIANAPOLIS

By GEORGE W. WADE

This study involves the agar plate count and the direct microscopic count of milk received from three sources, taken at weekly intervals, over a period of one year. It also includes recognition of the morphological types of bacteria found in milk, in an effort to determine the past history of the milks studied, and to determine the seasonal differences in the bacterial counts of this milk from each of the three sources.

Sedgwick and Batchelder (2) in the results of their bacteriological examination of Boston milk were among the earliest to bring to the public realization of the importance of dairy sanitation. The beginnings of certified milk in 1893 by Coit and Francisco (3) also involved the use of laboratory methods for controlling the number of bacteria present in milk and it was the city of Montclair, N. J., which first undertook regular bacteriological examinations of its milk supply. Probably the first work done on a state wide basis was that organized by Conn in Connecticut in 1908. The first committee of American Public Health Association was appointed in 1905 to standardize methods of control of milk. By the time the report was drawn up and adopted in 1910, 21 cities of the United States and Canada had adopted plans. In 1910 examinations in Baltimore, Philadelphia and Boston areas consisted of microscopic examination of centrifuge sediments for leucocytes and streptococci. In 1929-30, according to Standard Methods (1), 125 commercial laboratories analyzed more than 294,000 samples by the direct miscroscopic method in the United States and 2,500 samples in Canada.

The direct miscroscopic test first developed by Breed (1) has proved to be of immeasurable value on raw and pasteurized milk. It is the best means for determining the presence of excessive number of body cells in raw milk; it is a positive and reliable method for determining the typical intermingling of streptococci and cells in mastitis-infected milk; it also shows the presence of soil organisms, acid formers, colon and putrefactive types of bacteria.

The United States Government has set up regular rules as to the most effective means of drawing and handling milk (6). Where these regulations have been in effect, they have been of great help in improving the quality of the milk. Since in Indianapolis these rules are not in effect, it was believed that the method of handling milk in this city would lead to great differences in the quality of milk from different dairies. This was found to be true in a previous study by Miss Stanley (5) in this laboratory.

The direct microscopic method is rarely used by dairymen on pasteurized milk, but in many cases forms part of the tests of raw milk as it enters the dairy. Since each type of organism is an indicator of the conditions under which the milk is produced and distributed, a microscopic study of the smear reveals a history of the milk. The diplococci, paired spherical bacteria, are normal flora of milk; these are known as lactic acid bacteria. These forms in excessive numbers indicates slow cooling. The short streptococci, single or paried spherical bacteria in chains of 3-6 cells, are also lactic acid bacteria and in excessive numbers are likewise indicators of slow cooling. Long chains of spherical streptococci together with an excessive number of body cells, i. e., over 3,000,000 per cc, indicate mastitis.

Staphylococci and paried bacilli are indicators of unclean utensils. They originate in unclean surfaces and moist residues remaining on the surfaces of the cans between milkings, and from the fat and casein residues found in crevices and open seams of utensils and cans. Their presence in large numbers is an indication of dirty milking machines and cans. Mold fragments are seldom observed in fresh milk (4) but are commonly found around farms, and in milk which has come into contact with some surface containing a scum of sour milk solids. Their presence in large numbers is an indication of dirty cans, milking machine tubes, and connections.

Rods in clumps, individual rods and amorphous matter are indicators of dust, dirt, feed and other kinds of external contamination. These may be seen as long, heavy rods in chains, or in clumps; as short blocky rods also in chains or in clumps; and as long thin rods in chains. The sources of these forms and the mold which often accompanies them, are the flanks and udder of the cow, dust and dandruff from the cow, feed, wet milking, water, manure, etc. These bacteria often include thermoduric and thermophilic types. Bacteria

of the former group are not destroyed by pasteurization and those of the latter group grow abundantly at pasteurization temperatures.

PROCEDURE

Both agar and direct miscroscopic methods were used. In the latter, the object was to examine for types and numbers of each type of bacteria as well as total numbers. One pint bottle of milk, as delivered to the homes of consumers was obtained from each of three companies and taken to the laboratory for examination. This was done on each Saturday from December 3, 1938 to November 11, 1939. Companies delivering the milk are unaware of this experiment and are designated by the letters A, B and C.

The agar used for the plate method was prepared according to the formula recommended in Standard Methods (1). Sterile plates, agar and water blanks were used. The pipettes were kept in cleaning solution from one time of use until the next. Dilutions of 1:100 were found by experiment to be best and these dilutions were plated and incubated at 37° C for 67 hours. For the direct method, 0.01 cc of whole milk was taken in a calibrated pipette, deposited on a clean slide, and spread over an area of one square centimeter with a sterile needle. This was allowed to dry thoroughly, placed in xylol for five minutes to remove the fat, dried again, and dipped into 95% alcohol for five minutes to fix the material to the slide. After drying, the slide was dipped into a saturated aqueous solution of methylene blue for two to five seconds. Where necessary, the slide was destained in 95% alcohol.

A microscope with 15x ocular and 1.8 immersion oil objective was used for counting the bacteria. The drawtube was adjusted to give a field with a diameter of 0.146 mm making it possible to examine 1/600,000 part of a cubic centimeter of milk in each field. Thirty fields of each sample were observed for the number of body cells, bacterial clumps, diplococci, short streptococci (chains of eight or less), long streptococci (chains of more than eight), staphylococci, isolated cocci, isolated rods, rods in clumps, mold fragments, total number of bacteria and number of groups of bacteria.

RESULTS

The numbers of body cells, clumps, diplococci, short and long streptococci, staphylococci, isolated cocci, isolated rods, rods in

clumps, mold fragments, groups of bacteria and total bacteria per 30 fields are given in terms of monthly averages and seasonal averages in table I. In view of the dilutions and size of fields studied, each figure must be multiplied by 20,000 to secure the actual numbers per cc of original milk. Table II presents, in monthly and seasonal averages, a comparison of the counts by the plate and direct microscopic method.

Lactic acid bacteria and temperature. These organisms are diplococci and short streptococci. When large number of these bacteria are present it is conclusive evidence that the producer has failed to properly cool the milk, (4). The diplococci and the short streptococci followed the temperature curve except for Company A for the month of October, reaching an all time high of 2922 for the diplococci for 30 fields (1/20,000 cc). In short streptococci it was noticeable that Companies A and B kept their count down, with a low count of zero for Company A in November and of 5 for B in March and April. The lowest count of Company C was 12 for the month of February. When milk is cooled down to the optimum temperature for their growth (70° F) the streptococci have a tendency to become larger in size and arrange themselves in short chains. When milk is cooled carelessly (down to 80-85° F) these streptococci have a tendency toward a smaller size usually arranged in clumps which break up later to give the bacteria a scattered appearance. Poor cooling is a good explanation for the presence of excessive number of bacteria in the usual run of market milk (4).

Bacteria from unclean utensils. These organisms appear as isolated cocci, or as clumps of spherical bacteria (staphylococci), resembling bunches of grapes. Producers using milking machines which are not properly cleaned and sterilized are greatly troubled by this type of contamination. Staphylococci originate primarily from the fat and casein residues found in crevices and open seams of utensils and cans. They are abundantly found in dirty milk machines and cans (4). Except for an unexplained rise of Company A in January, the staphylococci followed the rise and fall of the temperature chart. All companies were high during the summer months, but Company C was very high, having twice the number of organisms per 30 fields as the second company. The all-time high here was Company C during months of June with 619; July with 635; August with 495; and September with 599. It is only during the

summer months that the staphylococci prove to be good indicators of dirty utensils.

Isolated cocci followed the chart of temperature except for the month of November when Company C rose to a high of 733 cells per 1/20,000 cc. This number (733) was the all-time high for the samples tested. Company C was high for six months; December with a count of 154; April, 43; May, 99; July, 613; August, 217; and November with 733. Company A had the lowest counts for the experiment with the following low counts per 1/20,000 cc: December, 42; May, 50; June, 83; August, 89; September, 63; and November, 48. Only in October with a count of 269 did this company (A) lead the other two companies. In interpreting the results we assume that the higher the staphylococci and isolated cocci count, the greater the lack of cleanliuess of utensils. The companies are thus rated: cleanest, Company A; dirtiest, Company C; and medium, Company B.

Bacteria from dirt. Belonging to this group are bacteria associated with dust, dirt, feed, etc. These can be recognized as long, heavy rods in chains or clumps. They are also associated with the above types of bacteria. Company C was predominantly high in isolated rods, throughout the entire experiment, but there was a tendency to follow the temperature curve. Company B was down throughout; Company A rose through the months of April and August. Company C was up enormously high in clumps of rods for the entire experiment while Company B was down throughout, with Company A rising from April to September, inclusive.

Mold fragments followed the temperature curve in all three companies with none present for January, February and March for any company. During the summer months the number rose moderately. Mold probably had its source with dirt, hut since the rise and fall of the number is closely related to the temperature curve, mold fragments are not good indicators of the amount of dirt present.

Many bacteria which gain entrance into the milk from the above named sources are of the thermoduric (heat-resisting) and thermophilic (heat-loving) types of organisms. In the former group, the bacteria are not destroyed by pasteurization, and in the latter group they grow abundantly at pasteurization temperatures.

Mastitis indicators. Body cells and long streptococci, when they appear together in large numbers, are indicators of mastitis. The

high number of long streptococci and of body cells were found in Company C in March. High counts of long streptococci are found in December for Company C, in February for Company B, and in October for Company A, but these high counts are not accompanied by correspondingly high counts of body cells. With these exceptions, the long streptococci count tends to increase from winter to summer and to decrease again in the fall, indicating that many long streptococci may be lactic acid bacteria which increase with temperature rises. Body cells in all three companies followed the air temperature curve with very little fluctuation, except for the months of December and January where the counts rise to 61 and 75 for the respective months in Company C.

A high leucocyte count should not always be interpreted as mastitis. Cows giving colostrum milk and stripper cows will show a high leucocyte count. The same is true of cows whose udders become bruised during any time of their lactation period, or whose resistance have become weakened by over-feeding, etc. Except for a high leucocyte count in Company B in October, the number does not vary much during the year in any of the companies. It is not easy to detect the presence of mastitis in composite samples of milk as received by the consumer. Individual cases should be tested.

Relation of Plate count to the total microscopic count and total group count. The plate and microscopic count both gave only estimates of the total number of bacteria present, but the latter is more accurate. This is true because the plate method counts only colonies, each one of which may have started from any number of individual cells. The microscopic method is also quick. Only a few minutes are required in the latter method to determine with what kind of milk one is dealiug. If the sample is not of high quality, the cause can be quickly determined and remedied. In order to learn anything from a plate count, two days must elapse for incubation, and by that time any undesirable milk which might be discovered will have been distributed to the consumer.

In all counts, the direct counts were much higher than the plate count, (table II) as would be expected. Not in all cases did the highest tally correspond in the same companies for the two methods, as in the plate count; Company A was high during the spring, summer and fall seasons, whereas the direct count was high only during the fall, dropping to the lowest of the three companies for each of the

other three seasons. In each case, however, the counts rose in correlation with the rise of temperature.

The ratio of the plate counts to the group microscopic counts are as follows: Company A, 1:150; Company B, 1:500; and Company C, 1:500. The ratio of the plate count to the individual microscopic counts are as follows: Company A, 1:110; Company B, 1:400; and Company C, 1:425. The higher the ratio, the higher is the number of dead organisms present to the number of living organisms. This is shown in Company A which had a lower microscopic count and a higher plate count than Company C which indicated that the number of living bacteria was higher in Company A. The high microscopic count of Companies B and C indicated poor past history, but this was fairly well covered up in the results of the plate count by pasteurization.

CONCLUSIONS

1. For the lactic acid bacteria Companies A and B held the low position and Company C was high throughout the year. All these counts rose in warmer weather.

2. Indicators of unclean utensils placed the companies in the following order: first, Company A; second, Company B; with Company C, third. Indicators here were good only during the summer months.

3. The rating of the companies by bacteria from dirt was: Company C, highest count, hence most dirt; Company B, lowest count, hence the least dirt; with Company A occupying the medium position.

4. Mold was not a good indicator of dirt, since the rise in the number of these organisms followed a constant pattern. No mold appeared during the months with low air temperatures, and the numbers of mold fragments were nearly identical during the months when the air temperatures were high. In no case did the number of mold fragments correlate in its rise and fall with the number of isolated rods and rods in clumps.

5. It is hard (or impossible) to detect mastitis in composite samples. There is one possible exception to this and that was Company C during March.

6. In the total counts the ratio of plate to direct microscopic count was lower in Company A and about equal in Companies B and C for both group and individual ratios.

7. There was no doubt as to which company ranked lowest in sanitary quality since C kept that position almost consistently. This milk evidently came from a dairy which was neglectful of sanitation in several respects.

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TABLE I

Number of organisms per 30 microscopic fields (1/20,000 cc of original milk) by direct microscopic count in terms of monthly and seasonal averages. First horizontal line for each month is Company A, the second line is B and the third is C.

Month	Body Cells	Clumps	Diplicocci	Short Streptococci	Long Streptococci	Staphylococci	Isolated Cocci	Isolated Rods	Rods in Clumps	Mold Fragments	No. of groups of bacteria per 30 fields	No. bacteria per 30 fields
December	55	38	56	12	42	17	42	7	18	..	156	212
	59	29	28	31	31	36	43	3	6	..	180	189
	61	99	95	30	1106	44	154	9	768	..	2200	2300
January	53	27	23	35	49	547	39	5	12	..	294	326
	51	15	20	13	31	44	29	1	..	..	136	151
	75	57	43	35	31	43	28	9	290	..	480	612
February	49	11	10	13	10	13	52	4	5	..	97	109
	41	28	28	27	120	46	36	2	1	..	261	290
	61	34	27	12	17	126	38	7	59	..	311	325
March	41	77	115	43	41	94	44	11	12	2	381	462
	44	8	9	5	12	5	27	2	..	4	64	72
	71	69	46	43	226	87	23	17	248	5	694	738
April	43	42	41	26	16	26	37	27	80	8	250	293
	43	11	16	5	6	6	33	3	..	3	65	81
	46	38	34	35	..	17	43	18	277	5	351	380

TABLE I—(Continued)

Number of organisms per 30 microscopic fields (1/20,000 cc of original milk) by direct microscopic count in terms of monthly and seasonal averages. First horizontal line for each month is Company A, the second line is B and the third is C.

Month	Body Cells	Clumps	Diplicocci	Short Streptococci	Long Streptococci	Staphylococci	Isolated Cocci	Isolated Rods	Rods in Clumps	Mold Fragments	No. of groups of bacteria per 30 fields	No. bacteria per 30 fields
May	56	61	60	31	30	53	50	40	85	2	350	411
	41	46	45	24	34	108	50	4	4	3	279	340
	39	167	165	25	42	113	99	55	426	5	890	1055
June	49	160	182	44	73	194	83	22	42	8	693	854
	60	114	143	49	142	336	225	8	15	3	927	1034
	56	363	443	468	192	619	187	128	399	8	2102	2465
July	82	261	293	123	151	192	88	43	43	4	935	1156
	62	225	708	152	275	294	40	16	14	2	1760	1985
	69	565	600	218	218	635	613	583	593	5	3561	4125
August	86	230	354	106	106	164	86	20	16	4	854	1085
	68	231	345	130	203	137	179	10	20	3	1029	1205
	55	786	1103	185	555	495	217	476	546	3	4057	4718
September	72	123	269	58	39	105	63	7	25	4	526	669
	55	78	100	36	47	81	64	2	3	3	356	426
	45	511	500	144	175	599	84	447	965	5	2798	3308

October	66	1569	2922	312	332	166	269	5	11	..	3987	5553
	107	78	112	38	42	104	107	2	..	2	375	483
	35	117	171	67	56	109	66	170	846	4	1491	1690
November	51	27	84	..	..	135	48	8	2	1	133	260
	37	25	34	17	..	35	120	6	..	4	207	232
	24	116	64	26	16	79	733	38	286	..	1266	1372
Winter	52	25	29	20	33	195	44	5	11	..	182	215
	50	24	25	23	60	42	36	2	2	..	195	210
	64	63	53	25	384	71	73	8	372	..	997	1079
Spring	46	60	72	33	29	57	43	26	59	4	327	388
	42	21	26	11	17	39	36	3	1	3	136	164
	52	91	81	34	89	72	55	30	317	5	645	724
Summer	72	217	376	94	100	183	85	28	33	5	660	1031
	63	190	398	110	206	256	148	11	16	2	1238	1408
	60	571	715	290	321	583	339	395	512	5	3240	3769
Fall	63	573	1095	156	123	135	126	6	12	1	1548	2160
	66	57	82	30	29	73	97	3	1	3	312	380
	34	238	235	79	82	262	294	218	699	3	1851	2123

TABLE II

Comparison of plate and direct microscopic counts in terms of monthly and seasonal averages. First horizontal line for each month is Company A, second is B and third is C.

Month	Plate Count	Direct Microscopic Count	
		Bacteria per cc	Groups per cc
December	24,750	4,232,000	3,048,000
	1,290	4,188,000	3,632,000
	26,740	63,750,000	25,796,000
January	14,287	6,275,000	5,980,000
	850	3,035,000	2,730,000
	7,962	10,750,000	10,015,000
February	32,575	2,975,000	1,950,000
	34,612	5,750,000	5,230,000
	56,462	6,145,000	5,595,000
March	55,938	9,440,000	7,595,000
	13,400	1,445,000	1,280,000
	45,712	15,255,000	13,625,000
April	54,012	7,560,000	5,855,000
	26,887	1,645,000	1,340,000
	47,850	7,812,000	7,025,000
May	25,850	8,230,000	7,000,000
	14,740	6,295,000	5,355,000
	48,150	21,150,000	18,220,000
June	67,250	17,080,000	14,620,000
	25,340	20,430,000	18,400,000
	53,650	41,850,000	39,176,000
July	214,140	23,052,000	18,696,000
	34,400	39,616,000	29,250,000
	195,720	82,498,000	67,224,000
August	64,650	21,700,000	17,909,000
	36,300	24,100,000	20,580,000
	135,050	81,860,000	76,145,000
September	113,040	28,320,000	13,376,000
	22,560	11,104,000	6,736,000
	150,200	66,172,000	55,956,000
October	530,400	83,575,000	60,535,000
	46,875	7,300,000	6,130,000
	86,550	25,350,000	22,360,000
November	30,600	6,280,000	5,700,000
	11,100	7,420,000	4,640,000
	38,100	27,440,000	25,120,000

TABLE II—(Continued)

Comparison of plate and direct microscopic counts in terms of monthly and seasonal averages. First horizontal line for each month is Company A, second is B aud third is C.

Month	Plate Count	Direct Microscopic Count	
		Bacteria per cc	Groups per cc
Winter	23,837	4,494,000	3,659,333
	12,230	4,357,666	3,864,000
	26,254	26,881,666	13,802,000
Spring	111,933	8,410,000	6,816,666
	18,342	3,128,333	2,568,333
	47,237	17,405,666	12,956,666
Summer	132,013	20,610,666	16,802,000
	32,013	28,048,666	22,743,333
	128,140	68,736,000	60,848,333
Fall	223,680	39,391,666	26,537,000
	26,845	8,608,000	5,835,333
	91,616	39,654,000	34,478,666